REMARKS

Claims 1-44 are all the claims pending in the application; claims 13-20 and 43-44 have been withdrawn from consideration; claims 1-12 and 21-42 have been rejected.

Claim 1 has been amended to correct a grammatical error.

Claims 5 and 22 have been amended to more clearly state that which Applicants regard as their invention. Support for the amendment of the claims to recite the CAG promoter as being operatively linked to the β-globin poly(A) sequence may be found in the specification at page 11, lines 15 to 19.

No new matter has been added. Entry of the amendment is respectfully requested.

I. Formal Matters

Applicants acknowledge receipt of the initialed and signed copies of the lists of references submitted with the Information Disclosure Statements in the present application on June 13, 2001 and August 9, 2002.

Applicants also note that two other Information Disclosure Statements have been filed in this application, on April 11, 2001 and November 26, 2002. Applicants respectfully request return of an initialed and signed copy of the lists of references accompanying these other Information Disclosure Statements.

II. Election

At page 2, first and second full paragraphs of the Office Action, the Examiner acknowledges Applicants' election of Group I, without traverse, in response to the Restriction Requirement mailed August 13, 2002.

The Examiner indicates that claims 1-12 and 21-41 are being examined. However, Applicants respectfully note that claim 42 should be included with the claims under examination (please see the Examiner's grouping of claims in the Restriction Requirement dated August 13, 2002). Thus, claims 1-12 and 21-42 are under consideration.

III. Claim Objections

At page 2 of the Office Action, the Examiner objects to claim 1 because it ends in a comma, not a period.

In response, Applicants include herewith an amendment to claim 1, making the necessary correction.

In view of the amendment to the claim, Applicants respectfully request reconsideration and withdrawal of this rejection.

IV. Claim Rejections - 35 U.S.C. § 112

In the last full paragraph at page 2 of the Office Action, the Examiner rejects claims 5, 22, 28-32 and 37 under 35 U.S.C. § 112, second paragraph, as being indefinite.

The Examiner's position is that these claims, either directly or indirectly, recite that the promoter is the CAG promoter, which is defined as comprising several sequences, including a splicing acceptor and a poly(A) sequence of rabbit β-globin. The Examiner believes that it is not accurate to refer to these components as parts of a promoter, since splice acceptors and poly(A) sequences are not normally parts of promoters.

In response, Applicants include herewith an amendment to claims 5 and 22, such that the promoter region is more clearly recited. As can be seen by the amendment, the poly(A)

sequence is no longer included as an element of the promoter, but instead recited as being operatively linked to the promoter.

With regard to the splice acceptor site, Applicants respectfully assert that this element is indeed considered to be part of the CAG promoter. A copy of an article by Oike et al. (*Blood*, 100(4):1326-1333 (2002)), is enclosed herewith. As explained therein, the CAG promoter is composed of a cytomegalovirus enhancer, a chicken β-actin promoter and a rabbit β-globin splicing acceptor (please see page 1327, left column, second full paragraph, lines 1-2). Thus, the splicing acceptor is considered to be part of the CAG promoter.

Oike et al. was published after the international filing date of the present application, but references the CAG promoter (reference #22) in another article, Niwa et al., *Gene*, 108:193-199 (1991), which was published before the international filing date of the present application. However, because Niwa et al. clearly does not describe the construction of CAG promoter, it does not teach or suggest any element of the claims of the present invention.

In view of the amendments to the claims, and the points discussed above, Applicants assert that the amended claims are definite as written and respectfully request reconsideration and withdrawal of this rejection.

V. Claim Rejections - 35 U.S.C. § 103

At pages 3 and 4 of the Office Action, the Examiner rejects all claims under 35 U.S.C. §103(a) as being unpatentable over Hardy (WO 97/32481) and Wahl et al. (WO 92/15694).

The Examiner's position is that Hardy teaches a cell that expresses recombinase Cre in functional form, and that Wahl teaches a FLP/FRT recombination system for expressing a gene

in an FLP dependent manner. The Examiner asserts that one of ordinary skill in the art wanting to express functional Cre stably in a cell without detrimental effects on the cell would readily employ the FLP/FRT recombination system of Hardy in the cell of Wahl and expect functional Cre to be expressed.

In response, Applicants state the following.

As seen from claim 1, the present invention relates to a cell expressing recombinase Cre in the presence of recombinase FLP in a FLP-dependent manner.

More specifically, as recited in claims 2 to 5, the present invention relates to a cell derived from a 293 cell that expresses Cre in a FLP-dependent manner and that expresses adenovirus E1A necessary for the replication of an adenovirus. The claimed cell is novel and unobvious particularly in that Cre is not permanently expressed, but rather it is transiently expressed when desired and when the adenovirus E1A gene is expressed. Thus, the claimed cell can remarkably reduce the cytotoxicity of Cre against the cell, and advantageously express Cre at a high level only when Cre is needed.

The claimed cell is extremely useful in a helper-dependent adenovirus vector system for constructing an adenovirus vector using a helper virus, as discussed below in more detail.

Specifically, the specification describes in detail, at page 10, line 25 to page 12, line 21, the construction of 293FNCre cells which are a preferred embodiment of the present invention. The 293FNCre cells have been constructed by introducing a CAG promoter, a recognition sequence of FLP (FRT), a stuffer sequence, a second FRT and the Cre gene, in this order from upstream, into the genome of 293 cells expressing adenovirus E1A, as recited in claims 4 and 5.

The 293FNCre cells of the present invention can express Cre at a high level transiently in a FLP-dependent manner when Cre is needed.

Thus, the 293FNCre cells are extremely useful in a helper-dependent adenovirus vector system for constructing an adenovirus vector for use in transfecting a foreign gene, using a helper virus.

As described in detail in the specification at page 12, line 22 to page 14, line 4, an adenovirus vector is constructed by a helper-dependent adenovirus vector system using 293FNCre cells together with a helper virus.

That is, an adenovirus vector having a foreign gene, but having only inverted terminal repeats (ITR) and packaging sequences and thus incapable of replicating by itself, is transfected into the 293FNCre cells. Simultaneously, a helper virus having a packaging sequence flanked by loxP sequences and capable of providing all functions necessary for the replication of the adenovirus vector having the foreign gene is also transfected into the 293FNCre cells.

In the 293FNCre cells, when introducing FLP protein or FLP gene into the cells, Cre is transiently produced at a high level in a FLP-dependent manner. The thus produced Cre excises the packaging sequence from the helper virus to render the helper virus itself unpackagable. As a result, the propagation of the helper virus is inhibited. However, because the helper virus can provide all functions necessary for the replication of the adenovirus vector having the foreign gene, the titer of the desired adenovirus vector having the foreign gene can be increased in the 293FNCre cells.

Consequently, from the 293FNCre cells, the objective adenovirus vector having the foreign gene is obtained at a high level, while the undesired contamination consisting of the helper virus is maintained at a quite low level.

Therefore, the 293FNCre cells are extremely useful in a helper-dependent adenovirus vector system for constructing an adenovirus vector for transfecting a foreign gene, using a helper virus.

Furthermore, as described in the specification at page 14, lines 1 to 4, in the method as discussed above when introducing FLP protein or FLP gene into the 293FNCre cells, an adenovirus vector is much more preferably used which expresses FLP protein and has a packaging sequence flanked by loxP sequences. This is because such an adenovirus vector has both of the functions of (1) an adenovirus vector expressing FLP protein in the 293FNCre cells thereby to express Cre in a FLP-dependent manner and (2) also a helper virus having a packaging sequence flanked by loxP sequences thereby to be inhibited in propagation but to provide all functions necessary for the replication of the adenovirus vector having the foreign gene.

In other word, the 293FNCre cells according to the present invention enable an extremely excellent helper-dependent adenovirus vector system for constructing an adenovirus vector for transfecting a foreign gene, using a helper virus.

The novelty of the claimed cell is particularly in the expression of Cre not constantly but transiently when desired, thereby to reduce the cytotoxicity of Cre, and is thus also extremely useful in a helper-dependent adenovirus vector system as discussed above in detail.

As to Hardy cited by the Examiner, Applicants note that the Examiner asserts that "Hardy also teaches that there is the possibility that if Cre is expressed all the time, there could be a negative selective pressure toward what the Cre is acting on, that functionally expressed Cre is needed, and that functional Cre expressing cells can be made by many ways that are well known in the art (page 14, lines 6-31, page 23, lines 11-23, and Example 3 starting on page 31)."

However, as discussed below in detail, Applicants respectfully assert that Hardy teaches nothing whatsoever of the negative selective pressure when Cre is constitutively expressed. Indeed, Hardy neither teaches nor even suggests the claimed invention whatsoever.

Hardy describes, at page 14, lines 6 to 31, a method for producing an *in vivo* packaged therapeutic rAd vector wherein an eukaryotic host cell is transfected with a therapeutic rAd vector and a helper rAd virus.

In the method, the helper virus is used to help the packaging of the therapeutic rAd vector, and has a packaging site flanked by loxP sites. Thus, the packaging site is excised from the helper rAd vector in the presence of Cre. As a result, the helper rAd vector is rendered unpackagable, thereby inhibiting the propagation of the helper virus.

However, noticeably, as described in Hardy at page 14, lines 26 to 31, only in the final infection step are host cells capable of expressing Cre used. In the method of Hardy, non-Cre expressing cells are preferably used in infection and transfection steps (see page 14, lines 29-30, also claims 17-22).

That is, in the infection and transfection steps, other than the final step, non-Cre expressing cells are transfected with both the therapeutic rAd vector and the helper rAd vector, so that the titers of virus particles of both vectors is sufficiently increased. Then, in the final step

for obtaining only the virus particles of the therapeutic rAd vector, Cre-expressing cells are transfected with both of the rAd vectors, thereby leading to the inhibition of only the packaging of the helper rAd vector.

As is clear from the above, the phrase "prolonged selective pressure will result in unwanted deletions in the helper rAd vector" at page 14, lines 30-31, of Hardy, indicates that if the Cre-expressing host cells are used in the steps other than the final step, the Cre expressed in the host cells will excise the packaging site from the helper vector (i.e., unwanted deletions) in the earlier steps, thus resulting in a reduced recovery of the helper rAd vector.

Thus, the term "selective pressure" in Hardy does not indicate the pressure on the action of Cre to inhibit or control Cre function, but indicates the action *per se* of Cre for deleting the packaging site to inhibit the propagation of the helper vector.

Hardy teaches nothing whatsoever of host cells *per se* expressing Cre in a controlled manner by themselves and expressing Cre transiently only when Cre is needed thereby to reduce the cytotoxicity of Cre, as claimed in the present application.

Hardy teaches at page 23, lines 11-23, nothing more than a general common knowledge for a Cre-expressing cell culture system. Also, Example 3 in Hardy only describes a general method for constructing a Cre-expressing host cell.

As is clear from the foregoing discussions, Hardy neither teaches nor even suggests cells *per se* expressing Cre in a controlled manner, and thus expressing Cre transiently only when Cre is needed, thereby reducing the cytotoxicity of Cre, as claimed in the present application.

Accordingly, Hardy neither teaches nor even suggests the claimed cells of the present invention which are novel particularly in expressing Cre not constantly but transiently only when

desired and expressing adenovirus E1A, and are thus extremely useful in a helper-dependent adenovirus vector system for constructing an adenovirus vector using a helper virus, as discussed above in more detail.

As to Wahl, the Examiner asserts in the Office Action that Wahl teaches a site specific gene activation system (specific protein expression) using FLP recombinase to control a gene product in a manner that depends on expression of recombinase FLP.

However, Wahl fails to teach use of such a system in the expression of Cre.

Further, Wahl describes nothing whatsoever of a helper-dependent adenovirus vector system for constructing an adenovirus vector using a helper virus.

As is clear from the foregoing discussions, there is no teaching or suggestion in Hardy to control the expression of Cre using cells which express Cre in a control manner by themselves.

Further, there is no teaching or suggestion in Wahl to apply the site specific activation system to the expression of Cre. Wahl teaches nothing whatsoever of a helper-dependent adenovirus vector system.

Therefore, there is no motivation, either in Hardy or Wahl, to combine the teachings of Hardy with those of Wahl to arrive at the present invention.

Moreover, the claimed cells of the present invention are novel particularly in expressing Cre not constantly but transiently only when desired and expressing adenovirus E1A, and is thus extremely useful in a helper-dependent adenovirus vector system for constructing an adenovirus vector using a helper virus. This characteristic of the claimed invention is not at all taught or even suggested anywhere in Hardy or Wahl.

Accordingly, Applicants assert that the presently claimed invention is unobvious over Hardy and Wahl, and therefore respectfully request reconsideration and withdrawal of this rejection.

VI. Conclusion

In view of the above, reconsideration and allowance of this application are now believed to be in order, and such actions are hereby solicited. If any points remain in issue which the Examiner feels may be best resolved through a personal or telephone interview, the Examiner is kindly requested to contact the undersigned at the telephone number listed below.

The USPTO is directed and authorized to charge all required fees, except for the Issue Fee and the Publication Fee, to Deposit Account No. 19-4880. Please also credit any overpayments to said Deposit Account.

Respectfully submitted,

SUGHRUE MION, PLLC
Telephone: (202) 293-7060
Facsimile: (202) 293-7860

WASHINGTON OFFICE

23373
PATENT TRADEMARK OFFICE

Drew Hissong
Registration No. 44,765

Date: April 21, 2003